can be studied, a model to minimise the time needed to achieve a reproducible mild degeneration with maintained cell viability was developed. Degeneration was induced by injecting trypsin into the center of the NP after a 48 hours pre-culturing period. The discs were then maintained in culture for up to 14 days. The discs maintained cell viability, and showed depletion of proteoglycans as early as 4 days after trypsin injection. Histology revealed cell proliferation and clustering in areas of the NP as the proteoglycan content decreased. Only a minor effect was found on the collagen-binding proteins, chondroadherin and fibromodulin, at 14 days.
Conclusions: We have developed an aseptic method to isolate bovine coccygeal discs from tissue freshly procured from the slaughterhouse and maintain them in long term organ culture. We have also developed a trypsin-induced IVD degeneration model without compromising cell viability. The proteoglycans are largely depleted as early as 4 days post injection, and cell proliferation and clustering reminiscent of disc degeneration is apparent at later times. Only minor evidence of degradation of chondroadherin and fibromodulin was found, suggesting maintenance of the collagenous framework of the tissue. This organ culture system has the potential to be a very useful tool to study the efficacy of IVD repair by biochemical stimuli.

179

DEMONSTRATION OF A NOVEL BIOSCAFFOLD SUITABLE FOR USE IN CARTILAGE TISSUE ENGINEERING THAT SUPPORTS CHONDROCYTE PHENOTYPE

A. Volk, M. Kim, **G.R. Dodge**
Univ. of Pennsylvania, Philadelphia, PA

Purpose: Producing an engineered biomaterial with native cartilage-like properties is crucial for functional cartilage repair. Many materials have been explored for their suitability for providing a scaffold supporting cell growth, including various polymers, peptide hydrogels, collagen, and agarose. Microbial biocellulose (BC), a natural polysaccharide that can be modified in both shape and resorbability, is of great interest for tissue engineering applications. We combined our self-aggregating suspension culture (SASC) approach with a free-floating BC scaffold and tested the ability of articular chondrocytes to interact and maintain their phenotype in culture. The scaffold was a biosynthesized form of cellulose produced by the bacteria *Acetobacter xylinum*, which provided a scaffold with significant fluid holding capacity, tensile strength, and shape retention. Such properties can provide an improved natural alternative to synthetic materials used for clinically relevant tissue repair applications. The objective of this study was to determine the biocompatibility of the BC with articular chondrocytes, as well as the sustained growth, viability and phenotype of the chondrocytes on and in various forms of BC.
Methods: Articular cartilage were removed from pigs or equine.Chondrocytes were plated at densities of 2×10^6 cells/ml to 2×10^7 cells/ml in poly-HEMA coated culture plates with free-floating bioscaffold as supplied by Xylos Corp. BC variables included matrix density and degree of chemcal modification. Some material was physically modified to determine if the cellulose fibers/lamellae arrangement influenced cell growth. Cultures were maintained for 3 weeks, and uncultured chondrocytes were evaluated as a control. Quantitative real-time PCR analysis was used to characterize expression (mRNA) levels of genes that are constitutively expressed in the extracellular matrix of articular cartilage, type II collagen, aggrecan, and cartilage oligomeric matrix protein (COMP), as well as genes encoding proteins that phenotypically uncharacteristic, such as type I collagen. Analysis of relative gene expression used the $2^{-\Delta\Delta Ct}$ method.
Results: Chondrocytes attached to the BC and produced matrix around and in the scaffold. This occurred according to the density of the cellulose, in which case cells either attached around the scaffold in the densely packed material or filled in the loosely packed cellulose. The results showed the BC was supportive of cell growth and facilitates matrix interactions. The rounded appearance and extensive matrix surrounding the cells were consistent with the morphology of chondrocytes in hyaline cartilage. Using conventional and quantitative real time PCR showed levels of mRNA for type II collagen, Aggrecan and COMP in parallel with uncultured chondrocytes and consistent with hyaline cartilage. Levels expression for type II collagen and aggrecan were higher compared to scaffold-free cultures, and approached levels close to uncultured cells.
Conclusions: This study was to determine the compatibility of BC scaffold with chondrocytes and whether this unique and readily modified polysaccharide would support chondrocyte phenotype and gene expression. Results demonstrated chondrocytes quickly interacted with the BC and the biocompatibility of the BC scaffold was supported by the microscopic assessments, cell morphology, and gene expression. Gene expression profiles were consistent in chondrocytes cultured with the bioscaffold as compared to uncultured and our SASC model. Gene expression analysis did not show a change towards a hypertrophic chondrocyte or fibroblastic-like phenotype. These results provide a basis for developing BC as a biomaterial for cartilage repair.

180

PHENOTYPIC ANALYSIS OF CELL SURFACE MARKERS AND GENE EXPRESSION OF HUMAN MESENCHYMAL STEM CELLS DURING MONOLAYER EXPANSION

L. Galois[1], C. Cournil-Henrionnet[2], Y. Wang[2], C. Huselstein[3], D. Mainard[1], D. Bensoussan[4], P. Netter[2], J. Stoltz[3], S. Muller[3], P. Gillet[2], A. Watrin-pinzano[2]
[1]Dept. of Orthopaedic Surgery, nancy, France; [2]UMR CNRS 7561, nancy, France; [3]UMR CNRS 7563, nancy, France; [4]UTCT, CHU Nancy, France

Purpose: Both chondrocytes and mensenchymal stem cells (MSCs) are the most used cell sources for cartilage tissue engineering. However, monolayer expansion to obtain sufficient cells leads to (1) the rapid dedifferentiation of chondrocytes and, concomitantly, (2) the reduced ability of MSCs to differentiate into chondrocytes, thus limiting their application in cartilage repair. The aim of this study was (1) to investigate the influence of the monolayer expansion on the phenotype and the gene expression profile of both cell types, and (2) to find the appropriate compromise between monolayer expansion and the retaining structure of chondrogenic characteristics.
Methods: Human chondrocytes, isolated enzymatically from femoral head slice, and human MSCs, derived from bone marrow, were maintained in monolayer culture up to passage 5. The expressions of cell surface markers (CD34, CD45, CD73, CD90, CD105, CD166) and several chondrogenic-related genes for each passage (P0 to P5) of those cells were then analyzed using flow cytometry and quantitative real-time PCR, respectively.
Results: Flow cytometry analyses showed that, during the monolayer expansion, some qualitative and quantitative regulation occur for the expression of cell surface markers. Chondrocyte expression pattern is similar to those for MSCs. A rapid increase in mRNA expression of type I collagen and aggrecan occurs whereas a significant decrease of type II collagen and sox 9 was observed in chondrocytes through the successive passages. On the other hand, the expansion did not induced obvious change in MSCs gene expression.
Conclusions: In conclusion, our results suggest that passage 2 might be the up-limit for chondrocytes in order to achieve their subsequent redifferentiation in 3D scaffold. Nevertheless, MSCs